12-2018

Vulnerability of Tri-Colored Bats (*Perimyotis subflavus*) to White-Nose Syndrome in the Southeastern United States

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VULNERABILITY OF TRI-COLORED BATS (*PERIMYOTIS SUBFLAVUS*) TO WHITE-NOSE SYNDROME IN THE SOUTHEASTERN UNITED STATES

A Thesis
Presented to
the Graduate School of
Clemson University

In Partial Fulfillment
of the Requirements for the degree
Master of Science
Wildlife and Fisheries Biology

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by
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December 2018

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ABSTRACT

Tri-colored bats (*Perimyotis subflavus)* have experienced significant population declines in the southeastern United States due to white-nose syndrome (WNS). The lack of information on the torpor patterns and winter activity in conjunction with the rapid decline from the disease has led to an increased effort in researching the hibernation physiology and wintering activity of *P. subflavus* and their response to WNS in southern hibernacula. To address this growing concern, we used temperature sensitive radio transmitters to examine torpor patterns in three geographically distinct states and acoustic detectors to monitor bat activity within a WNS-positive hibernaculum. Our specific objectives were 1) compare torpor patterns (torpor bout length, number of torpor bouts, arousal length, arousal frequency, and average skin temperature) between a WNS-positive and two WNS-negative sites, 2) examine the environmental factors that affect torpor patterns in the southeastern United States, and 3) investigate the environmental factors that affect *P. subflavus* winter activity within a hibernaculum.

To compare torpor patterns between WNS-positive and WNS-negative sites, we affixed temperature sensitive radio transmitters on *P. subflavus* in South Carolina (WNS positive), Mississippi (WNS negative), and Florida (WNS negative) during winters 2016-17 and 2017-18. We used linear mixed effects models to compare torpor between the WNS-positive and negative sites. We also tested the effects of environmental factors (hibernaculum temperature, ambient temperature, humidity), sex, and site on torpor parameters. *P. subflavus* average torpor skin temperatures ranged from 12.5ºC to 15.8ºC across sites and were within the optimal growth range of the fungus that causes WNS.

Torpor bout length, number of torpor bouts, and average torpor skin temperature did not differ between sites. However, males had longer torpor bout lengths than females. Bats in South Carolina had higher arousal frequencies than bats in Mississippi, and even though bats in Florida had higher arousal frequencies than those in South Carolina, the difference was not statistically different. Males aroused longer than females in the WNS negative sites but males and females in the WNS positive had similar arousals and potentially to minimize energetic costs in a diseased site. These findings suggest that *P. subflavus* are vulnerable to WNS among the sites because individuals' skin temperatures were with the fungus' optimal growth range (12ºC – 16ºC).

To investigate winter activity within a hibernaculum, we acoustically monitored bat activity from October 2016 – March 2017 and October 2017 – March 2018 at a WNS positive site in South Carolina. While *P. subflavus* were active throughout the winter, activity was generally low. We used generalized linear mixed models to test the effects of environmental factors on activity within the hibernaculum and used Akaike Information Criterion to evaluate support for a top model. Presence of bat activity was positively correlated with ambient temperature and negatively correlated with hibernaculum temperatures and bats were more likely to be active later in the day. While we detected bat activity throughout the winter, levels of bat activity were lower in mid to late winter than early winter and levels of bat activity were positively correlated with hibernaculum temperatures. This suggests that *P. subflavus* were able to detect small changes within the hibernaculum and if activity increases with warmer temperatures, individuals could potentially expend more energy during an energetically constrained time.

DEDICATION

I would like to dedicate this thesis in loving memory of my dearest grandfather, a devoted father and husband. Your memory I treasure, your devotion to your family I admire, and your wittiness I will forever miss.

ACKNOWLEDGMENTS

First and foremost, I would like to thank Dr. Susan Loeb for providing me with the opportunity to discover my passion for conservation through research. Her mentorship and teachings were invaluable to my professional development as a scientist. I would also like to thank my chair David Jachowski and my committee members, Dr. Brandon Peoples and Dr. Patrick Jodice, for their patience, feedback, engaging discussions, and encouragement throughout my graduate career. I would like to thank Andrea Kessler, Jennifer Hooper, and the rest of the administrative staff who have helped me immensely these past few years.

This project would not have been made possible without funding from the U.S. Fish and Wildlife Service, U.S. Forest Service Southern Research Station, South Carolina Wildlife Federation, and Clemson University PEGAS. I want to extend my gratitude to Eric Winters and volunteers who have been instrumental in providing me assistance in the field and the lab. I would like to thank my fellow cohort and the "bat lab" for providing me with a sense of community here at Clemson. I would like to thank Daniel Raitz, my partner in crime, my confidant and my dearest friend, and someone I cannot thank enough for his unending support. Finally, I would like to thank my mother who encourages me every day and who will continue to support me in the future.

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CHAPTER ONE

TORPOR PATTERNS OF TRI-COLORED BATS (*PERIMYOTIS SUBFLAVUS)* IN THE SOUTHEAST

1. Introduction

Heterothermic animals such as bats have the ability to reduce their metabolic rates, body temperatures, and heart rate (Geiser 2001) to conserve energy during periods of low food resources or cold temperatures. This behavior, known as torpor (Wojciechowski et al. 2007), allows bats to match their skin temperatures to the environment (5°- 15°C) and is interrupted by periodic arousals that are energetically expensive and during which metabolic rates and body temperatures rise to normothermic levels (39°C). Arousals are costly and can consume up to 80% of the total winter energy budget (Thomas et al. 1990). Hibernating bats must balance the duration of torpor and frequency of arousals to ensure they survive the winter and to support reproduction when they emerge in the spring (Thomas 1995, Czenze and Willis 2015 The importance of understanding torpor patterns in free-ranging bat populations has become crucial due to a fungal disease called white-nose syndrome (WNS) that affects hibernating bats during winter.

WNS is an epizootic disease that was discovered in New York in 2006 and is caused by the fungal pathogen *Pseudogymnoascus destructans* (*Pd*), a psychrophilic fungus that has killed over 6 million bats in the United States (USFWS 2018). *Pd* causes infections in the dermis layer of a bat's wing membrane and is associated with

disruptions in water balance and thermoregulatory processes, such as torpor (Hoyt et al. 2015). The infection can cause frequent arousals during winter hibernation leading to the premature depletion of fat stores, often resulting in death by dehydration or starvation (Cryan et al. 2010, Reeder et al. 2012, Warnecke et al. 2012). Infected individuals may not have the ability to replenish their fat stores, rehydrate, and thermoregulate at optimal hibernating temperatures (Lilley et al. 2016). *Pd* thrives in cold, damp places where the fungus persists in the environment (Flory et al. 2012) and grows fastest between 12.5°C and 15.8°C (Lilley et al. 2016, Verant et al. 2012), similar to hibernating bats' skin temperatures (Hoyt et al. 2015). Within the past decade, WNS has spread to 33 states and 7 Canadian provinces (USFWS 2018). Mortality rates in eastern hibernacula range between 30% and 90% (Frick et al. 2010) within the first few years of detection.

There are a few studies that demonstrate how various factors may drive torpor. Lab studies such as those by Boyles et al. (2007) analyzed microclimate selection in big brown bats (*Eptesicus fuscus*) and little brown bats (*Myotis lucifugus*) and found that individuals that weighed more selected warmer temperatures, thus requiring less energy to arouse. *M. lucifugus* also roost at higher humidity to minimize water loss (Thomas and Cloutier 1992) and Park et al. (2000) found that torpor bout duration was highly dependent on ambient temperatures in hibernating greater horseshoe bats (*Rhinolophus ferrumequinum).*

While torpor studies on *M. lucifugus* and *E. fuscus* exist, virtually no data are available on skin temperatures and torpor patterns of free ranging or lab-maintained tri-colored bats (*Perimyotis subflavus*) during winter. There are also no studies to date that

have documented the physiological effects of WNS on torpor patterns or how hibernaculum conditions can affect torpor of *P. subflavus* populations in the southeastern United States. *P. subflavus* were considered common throughout the southeastern United States, however, due to WNS, they have experienced >90% declines in southeastern hibernacula (Loeb, unpublished) and are a considered a species at risk in South Carolina (SCDNR 2018). Their range extends from southeastern Canada through most of the eastern United States, as far west as Colorado to the Yucatan (Fraser 2012, Adams et al. 2018). Considered solitary roosters and rarely found in large numbers, they prefer temperatures of 9-12°C and >80% humidity during hibernation (Briggler and Prather 2003). Since WNS has spread to most of the southeastern United States and *P. subflavus* are in decline, it is imperative we determine their torpor skin temperatures, torpor bout length, and arousals from both WNS positive and negative sites to establish baseline data and predict their susceptibility to WNS throughout their range.

We conducted the study in three geographically distinct sites that had established *P. subflavus* populations and selected one WNS positive site and two WNS negative sites that served as our controls. Our first objective was to test the hypothesis that torpor patterns of *P. subflavus* differed between WNS positive and negative sites in free-ranging populations. We predicted skin temperatures would be lower and that individuals would arouse more frequently (Reeder et al. 2012) and would have shorter torpor bouts to offset physiological demands of the disease in the WNS positive site. Our second objective was to test how environmental factors such as hibernaculum temperature, humidity, and ambient temperature affect torpor patterns in WNS positive and negative sites. We

hypothesized ambient temperature and hibernaculum temperature (Boyles et al. 2007) would be important factors in predicting torpor patterns such as torpor bout length and arousal length.

2. Materials and Methods

2.1 Study Area

We conducted our study in three *P. subflavus* hibernacula; our WNS positive site, Stumphouse Tunnel (ST), was located in Oconee County, South Carolina. One WNS negative site was located in Florida Cavern State Park (FCSP) in Jackson County, Florida and the other WNS negative site was located at the United States Naval Air Station Meridian (NASM), in Lauderdale County, Mississippi (Fig 1). Each site had historical populations of 100 – 300 individuals. Due to limited availability of large *P. subflavus* hibernacula, we were unable to conduct our study in both WNS negative and positive sites in each state.

ST was an abandoned railroad tunnel and was 493 m long, 5.2 m wide and 7.6 m high and composed of three chambers (A, B and C; Fig 2). Approximately halfway through the tunnel in section B, there is a 4.9 m wide x 6.1 m long airshaft that extends 18.3 m up to the surface (Oconee County 2018), creating constant airflow into ST. *P. subflavus* primarily roosted towards the back third of the tunnel in Chamber C (Fig 2) where hibernaculum temperatures and humidity remained relatively constant. The daily average ambient temperatures during the winter months (Oct – March) over the past 20

years were 4.94 – 15.39°C (avg 9.83 ± 6.08°C) (NOAA 2018). Prior to the arrival of WNS, ST housed over 300 *P. subflavus*. However, since the emergence of WNS in 2014-15, the population has declined by 91% (Loeb, unpublished data).

At FCSP we collected data from bats in Indian Creek Cave. The limestone cave was comprised of long, deep, and narrow underground passages and bats typically roosted 60 m from the entrance in one large room (Jennings and Layne 1957) where temperatures and humidity were constant. Historically, the average daily temperatures during the winter months (Oct – March) over the past 20 years were 7.58°C - 21.52°C (avg 14.62 ± 5.90°C) (NOAA 2018). In NASM, *P. subflavus* roosted in a series of culverts under the northern runways. The culverts are separated by two major streams, Ponta Creek and Big Reed Creek. Adjacent to the streams were narrow strips of mixed woodlands and pine habitats (Martin et al. 2005). The northern culverts were comprised of adjoining tunnels 3 m high, 3 m wide, and 250 m long. The average daily temperatures during the winter months (Oct – March) from the past 20 years were 5.29 – 18.80°C (avg 12.28 ± 6.45°C) (NOAA 2018).

2.2 *Field Data Collection*

We collected data during January – March 2016, November 2016 – March 2017, and November 2017 – January 2018 in ST. In FCSP, we collected data from January 2017 to March 2017 and in NASM we collected data January 2017 – March 2017 and November 2017 – January 2018. A total of 127 individuals were captured and fitted with temperature sensitive transmitters at the three sites (ST = 51, FCSP = 25, and NASM =

51; Appendix A). At each site we hand-captured available individuals while in torpor and recorded standard body measurements (forearm length, mass, sex) and calculated body condition index (BCI) (mass/forearm length) (Jonasson and Willis 2011). Bats at ST were examined with an ultraviolet light to assess WNS status (Turner et al. 2014). We clipped a small area of hair between the shoulder blades of each bat and attached a 0.40 g Holohil LB-2XT temperature-sensitive transmitter (Holohil Systems Ltd., Ontario, Canada) using surgical glue. Handling time did not exceed 15 minutes. In ST and NASM, we tagged individuals every 1-2 weeks to have continuous samples throughout the season. However, in FCSP all individuals were transmittered on 12 January 2017. We followed U. S. Fish and Wildlife Service decontamination protocols at all sites and all handling procedures were in accordance with the Clemson University Institutional Animal Care and Use Committee (AUP2015-078) and the U.S. Forest Service Institutional Animal Care and Use Committee (#2015-004).

We used Lotek SRX800-D1 dataloggers in each site to record skin temperature (T_{sk}) data. In ST, we set three dataloggers approximately 60 m apart (Fig 2) and attached $\geq$ 3 5-element Yagi antennas (n = 11) to each datalogger. At the two WNS negative sites, we deployed only one Lotek datalogger and two antennas. For all sites, the antennas covered the entirety of the primary roosting areas of *P. subflavus*. We programmed the dataloggers to scan for active transmitter frequencies for 20-30 seconds per antenna every 10-15 min. To acquire microclimate data, we placed Hygrochron iButtons (Maxim Integrated, San Jose CA USA) throughout the site to record temperature and relative humidity at 30 min intervals. We acquired hourly ambient temperature from the nearest

Remote Automated Weather Station (RAWS) at each site (Mesowest 2018) and downloaded Lotek data and replaced batteries every seven days to limit disturbance within the hibernacula.

2.3 *Definition of Torpor and Arousals*

Bats display two distinct patterns of torpor, shallow daily torpor and prolonged seasonal torpor (Geiser and Ruf 1995). In the lab, metabolic rates are used to differentiate torpor from normothermia (Willis 2007). However, in field settings, T_{sk}, which accurately reflects core body temperature, is used to define torpor patterns (Barclay et al. 1996). Using calibration curves from the manufacturer, we calculated values for T_{sk} using a second order polynomial equation (Britzke et al. 2010). We removed data points if T_{sk} was below 0ºC as we attributed this to transmitter malfunction or a low battery. Entry into torpor was evidenced by a reduction in T_{sk}, whereas active arousals were evidenced by a rapid increase of T_{sk} until it reached normothermic levels (T_{sk} > 28ºC) (Turbill and Geiser 2008). We calculated arousal length (AL) in minutes from the time an individual's T_{sk} rose to $\geq$ 22ºC to when the T_{sk} decreased < 22ºC (i.e., when a bat enters torpor; Table 1). Typically, individuals exhibit active arousals if T_{sk} exceeds 22ºC until temperatures reach 34 – 38 ºC (normothermic temperature) because bats thermoregulate during this period (Park et al. 2000). We did not include individuals that exhibited passive arousals (i.e., slowly increased T_{sk} over a period of time, > 100 mins) in calculations of AL. We calculated torpor bout length (TBL) in minutes as periods of low T_{sk} in between arousal events if T_{sk} remained constant > 30 mins. To control for different lengths of times that

bats were monitored, we calculated an arousal frequency index as number of arousal/number of transmitter days. Similarly, we calculated a torpor bout index as number of bouts/number of transmitter days, to control for the different lengths of time bats were transmittered or followed. Additionally, we calculated average T_{sk} during each torpor and arousal period.

2.4 *Statistical Analyses*

For our first objective, we hypothesized that TBL and AL would differ among sites due to WNS status. We predicted that individuals in the WNS positive site would exhibit shorter TBL than individuals in the WNS negative sites (Reeder et al. 2012). We predicted that individuals in the WNS positive site would have shorter AL (Jonasson and Willis 2011) and more frequent arousals (Cryan et al. 2010) than in the WNS negative site. We hypothesized that average torpor T_{sk} would differ between the sites and predicted that individuals in the positive site would have lower T_{sk} than individuals in the negative sites because individuals at the WNS negative sites were able to optimally hibernate at warmer temperature (Boyles et al. 2007). We hypothesized that average arousal T_{sk} would differ between sites and predicted that individuals in the WNS site would display higher arousal T_{sk} than in the negative sites. Additionally, we hypothesized that there would be differences in torpor patterns between males and females and predicted that females would have longer TBL and shorter AL than males because females would prioritize conserving energy for pregnancy in the spring (Jonasson and Willis 2011). Finally, we hypothesized arousal times would differ between the WNS

positive and negative sites and predicted that individuals in the WNS positive site would arouse randomly throughout the day while individuals in the WNS negative site would follow the natural circadian cycle and arouse closer to sunset or dawn. While there are conflicting studies of whether bats follow the circadian cycle and arouse near sunset (Czenze et al. 2013), we rationalized that winters in the southeast are warmer and the potential to forage increases and therefore, individuals would follow normal circadian cycles (Ehlman et al. 2013).

We used linear mixed effects models (LMM) with sex and site as our main effects and ambient temperature (T_A) as our covariate to analyze differences in TBL, AL, and average T_{sk}, among sites (Table 2). We tested our dependent variables (TBL, AL, average T_{sk}) for normality and transformed variables if they were not normally distributed. We accounted for pseudoreplicated individuals by using the individual bat as a repeated measure and our random effect (Czenze and Willis 2015), and treated site as a fixed effect. We used the Kruskal-Wallis test to compare torpor bout index and arousal frequency index among sites and sex. For each site, we performed a Rayleigh's test to determine if arousal times were randomly distributed (Park et al. 2000). We converted time of arousals to radians and calculated length of vector mean (r) for each site. We used a *z* test for large sample sizes as our test statistic for the Rayleigh's test (Batschelet 1981). We used a one-way ANOVA to compare T_A, T_H, humidity, and BCI among sites and used TukeyHSD post-hoc tests to compare levels of significance across sites. All analyses were conducted in R version 3.4.4 (R Development Core Team 2016) and

significance was assessed at the $P < 0.05$ level. We report mean $\pm$ SD and estimates, standard error, and *P*-values from our LMM.

For objective two, we developed 10 *a priori* hypotheses, a null (intercept only), and a global model using environmental variables such as hibernaculum temperature (T_H), ambient temperature (T_A), and humidity, as well as site and sex to predict TBL, AL, average torpor T_{sk}, and average arousal T_{sk} of *P. subflavus* (Table 3 and Table 4). We tested our covariates for normality and screened for multicollinearity using a correlation matrix and parameters with an $|r| \geq 0.70$ were not used in the same model. We then fitted LMM and ranked our models using Akaike's Information Criterion adjusted for small sample size (AICc; Burnham and Anderson 2004). We determined top models as those with $\Delta AICc \leq 2.0$, and we model averaged coefficients if closely competing models existed (Posada and Buckley 2004) and used the conditional average of the models to acquire parameter estimates of our top models. We determined significance of our parameter estimates by whether their upper and lower 95% confidence intervals overlapped 0. Our global model included all environmental variables, sex, and site. The sub-global models included main effects of either sex or site and the interactions of the environmental variables (Table 4). We predicted that as T_H decreased TBL would increase. We predicted that females would have higher TBL at lower T_H and T_A compared to males. We also predicted that T_A would have a negative effect on TBL and AL, whereas humidity would have a positive effect on TBL and AL. Finally, we predicted that T_H and T_A would positively affect arousal T_{sk}.

3. Results

Although 127 individuals were transmitted during 2016 – 2018 across all three sites, we collected data from only 70 individuals (ST = 42, FCSP = 12, and NASM = 16) due to equipment failures and flooding events. We obtained data from 41 males and 29 females totaling 1189 transmitter days across sites (ST= 21.07 $\pm$ 9.91 days/bat, FCSP = 21.75 $\pm$ 12.55 days/bat, and NASM = 2.68 $\pm$ 3.32 days/bat). In 2017 we re-transmittered three individuals (2 males and 1 female) in ST from the previous year. Mean T_A during data collection in ST was 7.73 $\pm$ 4.71°C, 16.48 $\pm$ 2.98°C in FCSP, and 12.19 $\pm$ 7.02°C in NASM and significantly differed across sites (F = 78.89, df = 2,236, $P < 0.0001$). Post hoc Tukey's HSD showed that T_A among sites all differed from each other (Tukey HSD, $P < 0.05$). Mean T_H was 12.55 $\pm$ 1.25 in ST, 13.88 $\pm$ 0.80 in FCSP, and 11.04 $\pm$ 4.52 in NASM and significantly differed (F = 50.54, df = 2,481, $P < 0.0001$) among sites. Post hoc Tukey's HSD showed that T_H among sites all differed from each other (Tukey HSD, $P < 0.05$). Hibernaculum humidity significantly differed between sites (F = 29.98, df = 2,481, $P < 0.0001$) and was 94.79 $\pm$ 8.15% in ST, 98.90 $\pm$ 1.15% in FCSP, and 87.07 $\pm$ 11.42% in NASM. Post hoc Tukey's HSD showed that humidity among sites all differed from each other (Tukey HSD, $P < 0.05$). Mean BCI was 0.170 $\pm$ 0.022 in ST, 0.179 $\pm$ 0.039 in FCSP, and 0.172 $\pm$ 0.0156 in NASM and did not differ (F = 0.548, df = 2,56, P = 0.58) among sites. Males had significantly lower (F = 9.18, df = 1,57, P = 0.004) BCI (0.16 $\pm$ 0.02) than females (0.18 $\pm$ 0.03)

Perimyotis subflavus entered into prolonged torpor at all three sites. We rarely observed individuals leaving the hibernaculum at ST and FCSP (i.e., missing data) but individuals potentially left the roost site at NASM because we lost signal indicating that individuals might have left the roost site. Torpor bout length ranged from 0.02 to 14.44 days across sites (ST = 4.2 $\pm$ 3.12 days, FCSP = 3.49 $\pm$ 2.12 days, and NASM = 2.58 $\pm$ 2.24 days) and did not differ significantly among sites (F = 1.82, df = 2,55, P = 0.17). The interaction between sex and site on TBL was not significant (F = 0.07, df = 2,51, P = 0.93) but there was a significant effect of sex (F = 6.48, df = 1,28, P = 0.017) and contrary to our hypotheses, males had longer TBL than females across sites (Fig 3). Even though there was a significant effect of T_A on TBL (F = 14.25, df = 1,134, $P < 0.001$), the correlation was small ($R^2 = 0.07$). The torpor bout index was 0.20 $\pm$ 0.11 in ST, 0.36 $\pm$ 0.52 in FCSP and 0.18 $\pm$ 0.27 in NASM and we found no significant differences among sites (KW H_2 = 4.33, df = 2, P = 0.12) or sex (KW H_2 = 2.52, df = 1, P = 0.11). Average torpor T_{sk} of individual bats ranged from 11.76°C to 18.95°C across sites (ST = 15.56 $\pm$ 1.44°C, FCSP = 16.01 $\pm$ 1.39°C, and NASM = 14.78 $\pm$ 2.21°C) and did not differ among sites (F = 1.37, df = 2,59, P = 0.26) or sex (F = 1.60, df = 1,42, P = 0.21). There was a significant positive effect of T_A on T_{sk} (F = 21.06, df = 1,197, $P < 0.0001$). However, the amount of variation explained by T_A was small ($R^2 = 0.08$).

Arousal length ranged from 30 mins to 593 mins across sites (ST = 94.21 $\pm$ 61.39, FCSP = 100.07 $\pm$ 160.53 mins, and NASM = 176.00 $\pm$ 221.59 mins). The natural log of AL did not differ across sites (F = 2.37, df = 2,57, P = 0.102). However, three arousals of bats in FL were extremely long and appeared to be outliers. When we removed the

outliers, we still found no significant differences across sites (F = 1.44, df = 2,59, P = 0.24) and T_A (F = 0.07, df = 1,200, P = 0.79), but found a significant effect of sex (F = 5.84, df = 1,34, P = 0.02). Males generally had higher AL than females among sites and there was no significant interaction between sex and site (F = 1.86, df = 2,59, P = 0.16). Even though the interaction between sex and site did not statistically differ, the least square means AL of males and females in each site showed that mean AL did not differ between sexes in the WNS positive site, but mean AL differed between sexes in the WNS negative sites (Fig 4). AF did not differ between males and females (KW H_2 = 1.43, df = 1, P = 0.23), but we found a significant difference among sites (KW H_2 = 8.09, df = 2, P = 0.02) (ST = 0.25 $\pm$ 0.14, FCSP 0.41 $\pm$ 0.50, NASM = 0.18 $\pm$ 0.27) (Fig 5). AF at NASM significantly differed from AF at FCSP (P = 0.03) and ST (P = 0.04). However, due to low sample size these results should be interpreted with caution. AF at FCSP did not significantly differ from AF at ST (P = 0.08, α = 0.05). We were only able to collect data from Jan – March 2017 at FCSP, therefore, these results could be misleading. Average arousal T_{sk} was 32.77°C $\pm$ 3.07 in ST, 31.13°C $\pm$ 2.65 in FCSP, and 28.13°C $\pm$ 3.83 in NASM. Average arousal T_{sk} significantly differed among sites (F = 11.87, df = 2,81 P < 0.001) and sex (F = 4.25, df = 1,55 P = 0.04). Bats in ST had higher T_{sk} than the negative sites and males had higher arousal T_{sk} than females. Arousal times of bats in all three sites did not differ from a random circular distribution: ST (z = 0.101, P = 0.17, n = 175), FCSP (z = 0.04, P = 0.91, n = 63), and NASM (z = 0.05, P = 0.94, n = 27).

Our top model for TBL carried 99% of the model weight and included interactions of all environmental variables, site, and sex (Table 5). None of the parameter

estimates had 95% confidence intervals that excluded 0. Our top model for AL included the interactions between sex, site, and all environmental variables and was the most parsimonious (Table 6). The parameters that were significant in our top model were the interaction between humidity and sex, and between T_A and sex (Table 7). As humidity increased, males spent less time in arousal than females. As T_A increased males had longer arousal lengths than females. Average torpor T_{sk} was best predicted by two models that included all environmental variables (T_H, T_A, and humidity) and the interactions between site and the environmental variables (Table 8). After we model averaged, the interaction between site and humidity (Table 9) was significant indicating that T_{sk} of individuals in NASM decreased with increasing humidity. Humidity also had a negative effect on T_{sk} in ST and FCSP, but the relationship was not statistically significant. Site (ST) was also a significant parameter in our top models (Table 9), which suggests that individuals in ST had higher average torpor T_{sk} relative to FCSP and NASM. Our top model for average arousal T_{sk} included the interactions between site and sex (Table 10). However, none of the parameters estimates had a 95% confidence interval that excluded 0.

4. Discussion

Our results demonstrated that *P. subflavus* T_{sk} occurred within *Pd* optimal growth range (12.5°C – 15.8°C) at all sites. T_H also occurred within the Pd optimal growth range at all sites; NASM temperatures fluctuated more frequently but still were within the

range. *P. subflavus* entered into deep torpor for a maximum of 14 days and an average of four days at our study sites in the southeastern United States. Maximum TBL results from our study were similar to the average TBL for *M. lucifugus* (Czenze et al. 2013) found in the Northeastern U.S. *P. subflavus* had periodic arousals and the number of arousals were similar compared to studies on *M. lucifugus* (Lilley et al. 2016). We expected AF to significantly differ between the WNS positive and negative sties (Reeder et al. 2012, Warnecke et al. 2012) and while AF of individuals at ST differed from those at NASM (WNS negative) we were only able to record an average of two days of data from individuals in NASM and results from this analysis should be interpreted with caution. The AF index showed that individuals in FCSP (a WNS negative site) had more arousals than individuals in the positive site (ST). Additionally, we found AL differed between males and females where males had longer arousals than females. Finally, we did not find any evidence that individuals aroused at a particular time of day.

4.1 *Environmental Conditions*

Environmental conditions such as humidity, T_A, and T_H differed among sites. However, the hibernacula temperatures at all three sites were within the normal range for *P. subflavus* winter roosts (Briggler and Prather 2003) and the variation was most likely due to structural differences. ST allowed for more airflow through convection in the tunnel creating colder temperatures during the winter months compared to NASM and FCSP. ST is at a higher elevation (509 m) in the foothills of the Blue Ridge Mountains and farther north than the other sites. The elevation and geographic location of ST could

create colder temperatures during winter compared to the limestone caves in FCSP, which were located in the panhandle of Florida and culverts in NASM which were exposed to greater temperature fluctuations.

4.2 *Torpor*

Unlike other studies that have found differences in torpor bout length between *Pd*-exposed big brown bats and controls (Moore et al. 2018) our results demonstrated that while individuals in the WNS positive site exhibited deep torpor, the length of torpor bouts did not statistically differ from the negative sites. We also did not find any evidence to support our hypotheses that number of torpor bouts and average torpor T_{sk} differed among sites. Individuals in FCSP and NASM had similar TBL and number of bouts to individuals in ST which suggests use of similar hibernation strategies (i.e., prolonged deep torpor and similar torpor T_{sk}) across WNS positive and negative sites. Even though TBL did not differ among sites, there was a significant difference in TBL between males and females. Contrary to our hypotheses, males had longer torpor bouts than females and this did not vary with site suggesting the strategy to conserve energy is the same for males across all sites. However, males also had lower BCI than females. Thus, the longer torpor bouts that we observed is consistent with one of the hypotheses in Humphries et al. (2003) that states that individuals with lower BCI should use longer, deeper torpor bouts than individuals that enter hibernation with greater BCI. NASM had the lowest number of transmittered days therefore results from the site should be carefully interpreted.

While average torpor T_{sk} did not differ among sites, there was a negative relationship between humidity and T_{sk} at NASM. Humidity is often associated with evaporative water loss (EWL). Thomas and Cloutier (1992) demonstrated that bats exposed to drier climates exhibit greater water loss during torpor and decrease TBL. The relationship between humidity and T_{sk} in ST and FCSP was also negatively correlated but the relationship was not statistically significant. Therefore, the significant negative relationship between humidity and T_{sk} at NASM could indicate that humidity in the culverts was more variable than in the cave or tunnel. ST was a significant predictor in our top model, however, the positive relationship between ST and average T_{sk} could be misleading due to potential collinearity in our variables. While we concluded our variables were not correlated in our correlation matrix, there could be underlying correlation among the environmental variables which could explain the positive relationship between ST and average T_{sk}.

4.3 *Arousals*

Our results are not consistent with other studies (Reeder et al. 2012) that have found increased arousals in WNS affected individuals compared to WNS non-affected individuals. While we found that AF differed between ST and NASM, we only had on average two transmittered days per bat as compared to the average 21 days in ST. Even though bats at FCSP displayed more frequent arousals than individuals in ST, the relationship was not statistically significant. T_H and T_A at FCSP were warmer than at ST and therefore it is possible that individuals left the cave and foraged during warmer

nights. In our data, we had a few hours to days of missing torpor and arousal data. This could be attributed to poor signal between the transmitter and the data logger or the individual could have moved to a different area in the roost and our data loggers failed to pick up the signal. The missing data could also indicate that individuals left the roost site. However, only a few individuals had missing data. *P. subflavus* at FCSP also shared the hibernaculum with a colony of *Myotis austroriparius* that were very active. The high activity of *M. austroriparius* could have disrupted *P. subflavus* torpor and arousal patterns along with the higher T_H and T_A in FCSP.

We did not find support for our hypothesis that AL differed across sites; instead we found that AL differed between sexes. Males had longer AL than females in the WNS negative sites however, in the WNS positive site AL did not differ between males and females. We expected to find that males would have longer AL than females in the WNS positive site because females should minimize energy expenditure more than males due to expected costs of pregnancy post hibernation (Jonasson and Willis 2011). Shorter AL in males suggests that they were conserving energy similar to females, most likely due to WNS infection. Further, males had lower BCI than females, which could have added constraints in their energy budget (Humphries et al. 2003, Jonasson and Willis 2011) and ST had colder T_H compared to FCSP, which could have resulted in shorter arousal times since it is energetically beneficial to arouse at warmer temperatures with lower body mass (Boyles et al. 2007). Another plausible reason for shorter arousals by males in ST could be related to humidity. Humidity and sex were significant parameters that predicted AL and AL was negatively correlated to humidity for males. Males aroused longer than

females in the WNS negative sites and potentially incurred a greater water deficit through longer arousals (Thomas and Cloutier 1992, Thomas and Geiser 1997). Conversely, males in ST had shorter AL than males in the negative sites therefore, it is possible that individuals decreased AL to minimize EWL (Willis et al. 2011).

4.4 *Conclusions*

While empirical evidence is lacking on whether *P. subflavus* behavior and physiology has altered due to WNS, we conclude from this study that AL differed between the positive and negative sites and we can infer this difference may be due to WNS. Boyles et al. (2007) found empirical evidence that *M. lucifugus* that roost at warmer T_H have higher survival than individuals that roost at 2°C. Therefore, we need to acquire additional data on the torpor patterns and microclimate conditions of *P. subflavus* to make assumptions on their vulnerability to WNS throughout their range. Based on our results we know *P. subflavus* T_{sk} in hibernacula as far south as Florida were within the optimal *Pd* growth range (12°C – 16°C). Torpor patterns such as TBL, number of torpor bouts, torpor T_{sk}, did not differ between the sites. Thus, if FCSP and NASM become *Pd*-positive we could witness similar declines as in ST. Future studies can compare our data to the roosting site temperatures of both affected and unaffected sites of *P. subflavus* to better understand their vulnerability and thereby predict which sites may be affected by WNS.

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Table 1. Descption of torpor and arousal paramters.

Torpor Patterns	Definition
Torpor bout length (min)	Calculated in minutes from when an individual entered torpor and the T_{sk} was $\leq$ 22ºC to when T_{sk} $\geq$ 22ºC.
Number of torpor bouts	Number of torpor bouts during an individual's transmittered life.
Average skin temperature	Average T_{sk} during each torpor bout and arousal.
Arousal length (min)	Calculated in minutes from when an individuals' T_{sk} was $\geq$ 22ºC and T_{sk} reached normothermic temperatures to when the T_{sk} decreased below 22ºC or entered into torpor.
Arousal frequency	Number of arousals during an individual's transmittered life.
Arousal time	The time of day (24hr) an individual aroused during its transmitted life.

Table 2. Covariates used in linear mixed effects model for testing the differences in torpor bout length average torpor skin temperatures, arousal lengths, and average arousal skin temperatures.

Parameter	Description
Bat	Transmittered individual that was used as random effect
Site	White-nose syndrome positive site at Stumphouse, South Carolina or one of two white-nose syndrome negative sites at NASM, Mississippi, and Florida Caverns State Park, Florida.
Sex	Male or female
Ambient Temperature	Mean temperatures collected hourly at each site and downloaded from Remotely Automated Weather Station (RAWS) website.

Table 3. Covariates used for *a priori hypotheses* to predict torpor patterns.

Variable	Description
Individual Bat	
Sex	Male or female
Environmental Variables	
Humidity (Humid)	Average hibernaculum humidity collected at 30-minute intervals
Hibernaculum Temperature (T_H)	Average temperature within the hibernaculum collected at 30-minute intervals
Ambient Temperature (T_A)	Mean temperatures collected hourly at each site and downloaded from Remotely Automated Weather Station (RAWS) website.
Study Area	
Site	White-nose syndrome positive site at Stumphouse, South Carolina or one of two white-nose syndrome negative sites at Naval Air Station Meridian, Mississippi, and Florida Caverns State Park, Florida.

Table 4. List of *a priori models* for torpor bout length, average torpor skin temperatures, arousal length, and average arousal skin temperature.

Models
Torpor Bout Length and Arousal Length
Environmental
1. β1(TH) + β2(TA) + β3(Humid)
Site
2. β1(Sex)
Sex
3. β1(Site)
Interactions
4. β1(Site*TH) + β2(Site*TA) + β3(Site*Humid)
5. β1(Sex*TH) + β2(Sex*TA) + β3(Sex*Humid)
6. β1(Site*Sex)
Sub-Global
7. β1(TH) + β2(TA) + β3(Humid) + β4(Site) + β5(Sex)
Global
8. β1(Site*TH) + β2(Site*TA) + β3(Site*Humid) + β4(Sex*TH) + β5(Sex*TA) + β6(Sex*Humid) + β7(Site*Sex) + β8(TH) + β9(TA) + β10(Humid) + β11(Site) + β12(Sex)
Intercept
9. ~

Table 5. Akaike information criterion results for torpor bout length.

Torpor Bout Length			
Model	K	ΔAIC_C	Wi
Global	28	0.00	0.99
β1(Site*TH) + β2(Site*TA) + β3(Site*Humid)	11	94.95	0.000
β1(Sex*TH) + β2(Sex*TA) + β3(Sex*Humid)	11	152.93	0.000
β1(TH) + β2(TA) + β3(Humid) + β4(Site) + β5(Sex)	7	166.86	0.000
β1(Site*Sex)	5	184.03	0.000
β1(TH) + β2(TA) + β3(Humid)	5	213.23	0.000
Intercept	~	262.68	0.000

Table 6: Akaike information criterion results for arousal length.

Arousal Length			
Model	K	ΔAIC_C	Wi
Global	28	0.00	0.999
β1(Site*TH) + β2(Site*TA) + β3(Site*Humid)	11	57.90	0.000
β1(Site*Sex)	5	87.63	0.000
β1(Sex*TH) + β2(Sex*TA) + β3(Sex*Humid)	11	87.64	0.000
β1(TH) + β2(TA) + β3(Humid) + β4(Site) + β5(Sex)	7	95.20	0.000
β1(Site)	3	114.01	0.000
Intercept	~	126.67	0.000

Table 7: Top model parameter estimates for arousal length.

M4	Estimate	Lower	Upper
Humid	-17.67	-137.51	102.18
Humid: Sex M	-51.53	-83.59	-19.48
Humid: Site Mississippi	-17.53	-140.71	105.65
Humid: Site South Carolina	66.96	-54.42	188.33
Sex M	26.88	-24.50	78.27
Sex M: Site Mississippi	52.39	-43.04	147.82
Sex M: Site South Carolina	-2.99	-62.85	56.86
Sex M: TA	26.85	1.62	52.08
Sex M: TH	11.02	-11.22	33.25
Site Mississippi	-72.27	-159.69	15.15
Site Mississippi: TA	-2.34	-47.79	43.11
Site Mississippi: TH	0.83	-49.85	51.52
Site South Carolina	8.59	-47.21	64.38
Site South Carolina: TA	-25.82	-59.13	7.49
Site South Carolina: TH	-11.98	-61.47	37.52
TA	-1.23	-30.28	27.82
TH	15.02	-31.65	61.69

Table 8. Akaike information criterion results for average torpor skin temperature.

Average Torpor Skin Temperature			
Model	K	ΔAIC_C	Wi
β1(Site*TH) + β2(Site*TA) + β3(Site*Humid)	11	0.00	0.570
β1(TH) + β2(TA) + β3(Humid)	5	0.90	0.363
Global	28	5.73	0.032
β1(TH) + β2(TA) + β3(Humid) + β4(Site) + β5(Sex)	7	6.34	0.024
β1(Sex*TH) + β2(Sex*TA) + β3(Sex*Humid)	11	7.90	0.011
Intercept	~	17.77	0.000

Table 9: Top model parameter estimates for average torpor skin temperature.

M4 and M1	Estimate	Lower	Upper
Humid	2.00	-1.85	5.86
Humid: Sex M	0.29	-0.98	1.57
Humid: Site Mississippi	-3.95	-7.43	-0.47
Humid: Site South Carolina	-3.00	-6.26	0.25
Sex M	-0.19	-1.22	0.84
Sex M: Site Mississippi	1.40	-2.39	5.18
Sex M: Site South Carolina	-0.01	-1.60	1.58
Sex M: TA	0.33	-0.49	1.15
Sex M: TH	-0.08	-0.83	0.67
Site Mississippi	4.32	-2.20	10.85
Site Mississippi: TA	-5.62	-14.89	3.66
Site Mississippi: TH	2.96	-1.68	7.61
Site: South Carolina	1.85	0.05	3.66
Site South Carolina: TA	-0.49	-1.66	0.68
Site South Carolina: TH	0.32	-1.05	1.70
TA	0.47	-0.55	1.48
TH	0.09	-1.02	1.20

Table 10: Akaike information criterion results for average arousal akin temperature.

Average Arousal Skin Temperature			
Model	K	ΔAIC_C	Wi
β1(Site*Sex)	5	0.00	0.825
β1(Site)	3	3.55	0.140
β1(TH) + β2(TA) + β3(Humid) + β4(Site) + β5(Sex)	7	6.47	0.032
Global	26	12.26	0.002
β1(Site*TH) + β2(Site*TA) + β3(Site*Humid)	11	12.97	0.001
Intercept	~	24.30	0.000

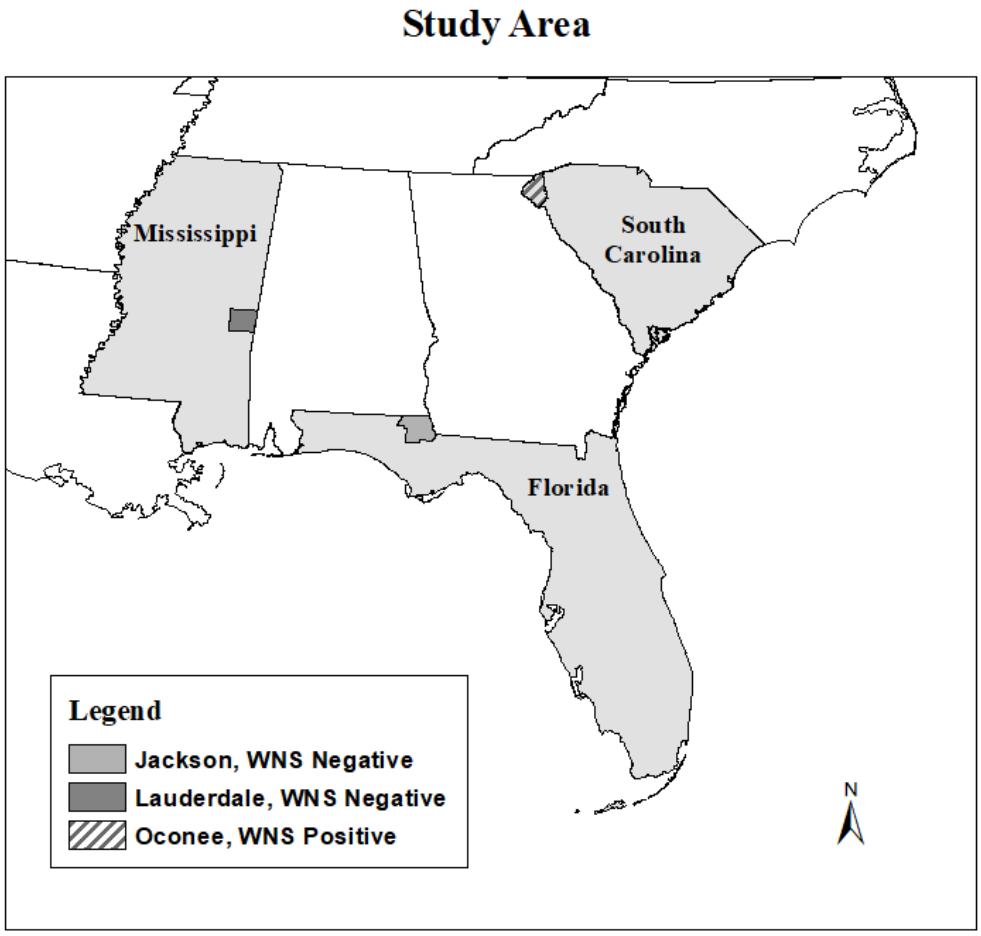

Figure 1. Map of study area – Counties of the study site are highlighted in gray. The striped color highlights the white-nose positive site while the lighter shades of gray highlights the white-nose negative counties.

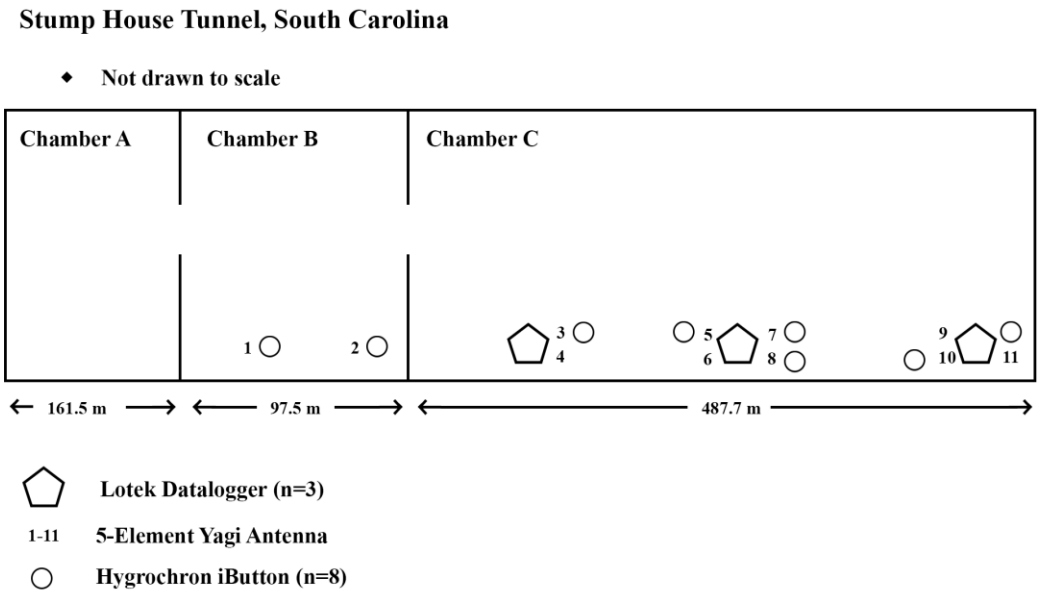

Figure 2. Schematic of Stumphouse Tunnel, South Carolina (white-nose syndrome positive site) and equipment set-up.

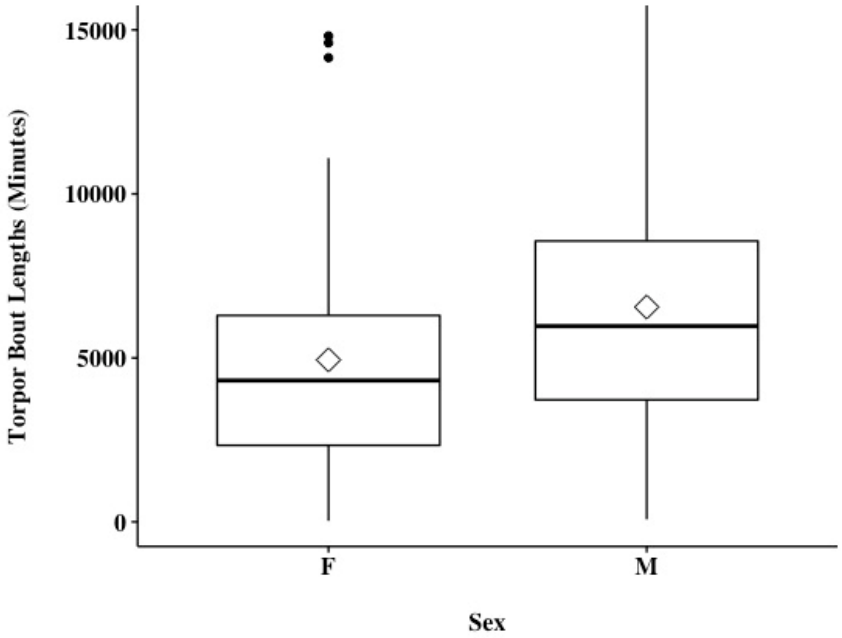

Figure 3. Boxplot of torpor bout length of males and females collected from South Carolina (January 2016 – January 2018), Florida (January – March 2017), and Mississippi (January – December 2017). Diamonds indicated average torpor bout length.

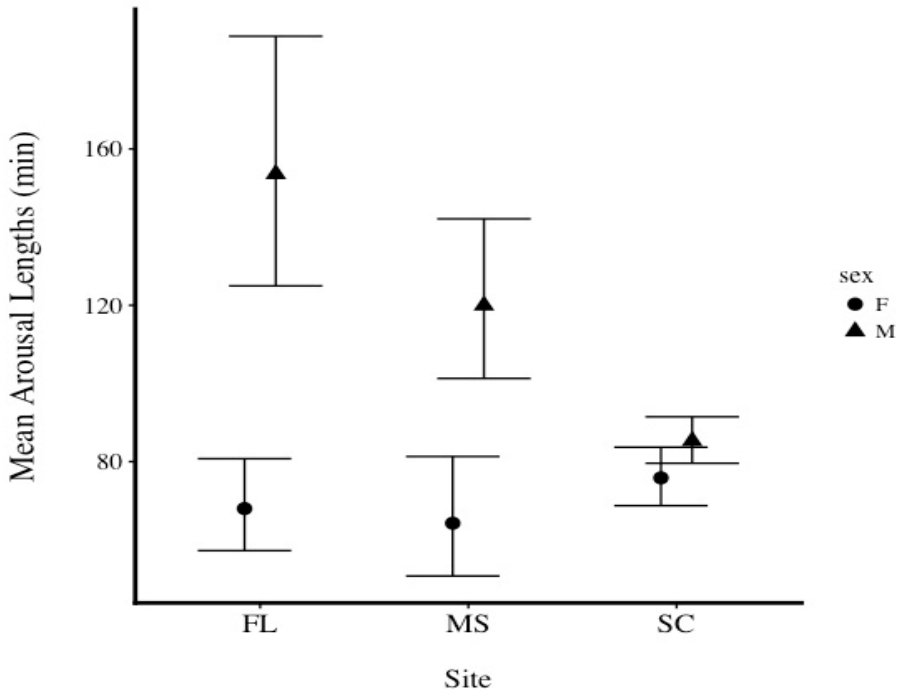

Figure 4. Mean arousal lengths of males and female tri-colored bats in Florida (Florida Cavern State Park, WNS negative), Mississippi (Naval Air Station Meridian, WNS negative), and South Carolina (Stumphouse Tunnel, WNS positive).

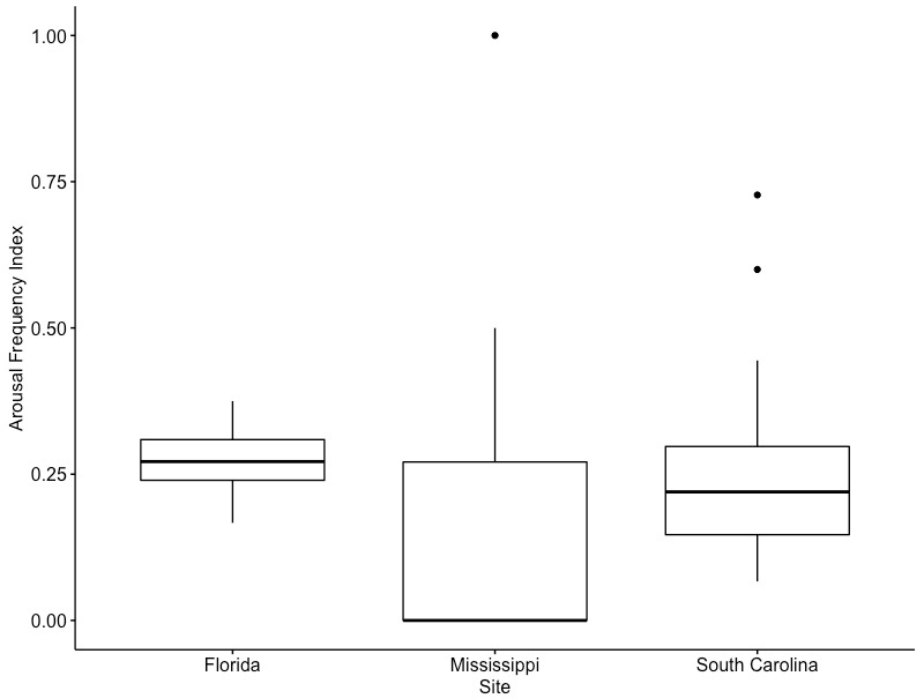

Figure 5. Arousal Frequency index across sites. Significance were assessed when $P < 0.05$. Florida and Mississippi differed to each other ($P = 0.03$) and South Carolina and Mississippi differed to each other ($P = 0.04$) however, Florida and South Carolina did not differ to each other ($P = 0.06$, $\alpha = 0.05$).

CHAPTER TWO

EFFECTS OF ENVIRONMENTAL FACTORS ON TRICOLORED BAT (*PERIMYOTIS SUBFLAVUS)* WINTER ACTIVITY

1. Introduction

Torpor in hibernating bat species is not continuous; it is interrupted by periodic energetically expensive arousals when individuals increase their body temperatures to reach euthermic temperatures (Thomas et al. 1990, Thomas and Cloutier 1992). Periodic arousals are energetically expensive, and arousals throughout the winter can cost an individual up to 80% of its total winter energy budget. Bats arouse periodically to offset physiological responses (e.g., waste excretion or oxygen build up), minimize evaporative water loss and dehydration (Thomas and Cloutier 1992), or follow a circadian rhythm and time their arousals to sunset (Malan 2010, Körtner and Geiser 2000). While scientists have studied the energetic costs of arousals, the subsequent causes for activity after arousals are understudied (Daan 1973, Ehlman et al. 2013).

Activity outside of the hibernaculum by bats during winter is relatively common (Boyles et al. 2006) and foraging (Avery 1985), drinking (Thomas and Cloutier 1992), mating, or roost switching (Whitaker and Rissler 1992) are commonly reported reasons why bats could be active during winter (Park et al. 1999). However, activity within a hibernaculum has not been fully explored. Common reasons to move within a hibernaculum are for copulation (Barclay et al. 1979) or to locate more suitable microclimates (Twente 1955).

Mechanisms for arousal and subsequent activity could be related to environmental factors. Park et al. (1999) found significant relationships between activity patterns, hibernacula temperatures, and sunset times. Activity is sometimes associated with barometric pressure (Paige 1995). However, contrasting results from several studies have hindered a thorough understanding of the relationship between barometric pressure and bat activity (Bender and Hartman 2015). For example, Berkova and Zukal (2010) found a positive relationship between late winter nightly activity and mean barometric pressure. Conversely, Czenze and Willis (2015) found negative relationships between barometric pressure and winter activity outside a hibernaculum. These conflicting patterns may have been the result of seasonal or geographical differences among studies, or varying methods for analyzing barometric pressure (mean nightly versus daily changes in barometric pressure). Ambient temperature is also associated with activity. Meyer et al. (2016) found a positive correlation between bat activity and outside ambient temperature but found no significant relationships between winter bat activity and hibernaculum temperature or barometric pressure. It is important to note that past studies only measured bat activity outside a hibernaculum and to our knowledge, effects of barometric pressure and temperature on bat activity within a hibernaculum have yet to be explored.

Bats populations in North America have been decimated from a disease called white-nose syndrome (WNS). WNS is an epizootic disease that was first discovered in New York in winter of 2006 and has since spread to 33 states and 7 Canadian provinces (USFWS 2018). WNS affects bats during winter hibernation and causes frequent arousals which can prematurely deplete fat stores resulting in mortality (Warnecke et al. 2012).

While bats typically arouse during winter hibernation to maintain physiological functions (Czenze et al. 2013) and can potentially leave the hibernaculum on warmer nights, WNS infected individuals have been reported to exit the hibernaculum even when temperatures are not ideal (Turner et al. 2015, Bernard and McCracken 2017). Currently, four species (*Myotis septentrionalis, Myotis sodalis, Myotis lucifugus,*and *Perimyotis subflavus*) have suffered population declines from the disease with many more that display positive clinical signs of WNS (Hoyt et al. 2015).

While data on wintering bat activity exist (Whitaker and Rissler 1992, Bernard and McCraken 2017), virtually no data are available on the activity patterns or the environmental conditions that affect *P. subflavus* activity within a hibernaculum. *P. subflavus* were once common and were found from southeastern Canada throughout most of the eastern United States, as far west as Colorado to the Yucatan (Adams et al. 2018). South Carolina is one of several WNS-positive states and there have been declines of > 90% of *P. subflavus* in the northwestern portion of the state. While WNS is an imminent threat to *P.* subflavus, increasing temperatures or climate change could also affect winter activity patterns of bats in the future (Sherwin et al. 2013, Humphries et al. 2002). Winter activity is often correlated with ambient temperature (Whitaker and Rissler 1992, Park et al. 2000, Lausen and Barclay 2006). Therefore, warming temperatures could impact future foraging patterns and activity. Monitoring bat activity within a hibernaculum could give us a better understating of what environmental factors affect activity during wintering months especially if future climates are predicted to be warmer in the southeast.

Our objective was to test how environmental factors such as hibernaculum temperature, humidity, barometric pressure, and ambient temperature affected *P. subflavus* activity within the hibernaculum. We hypothesized ambient temperatures and hibernaculum temperatures would be important factors in predicting activity (Meyer et al. 2016) and predicted that activity would increase with increasing ambient temperatures. We predicted that activity would increase as hibernaculum temperature increased because *P. subflavus* are thought to prefer higher temperatures within a hibernaculum (Briggler and Prather 2003) and cost of arousals would be less (Boyles et al. 2007). We hypothesized *P. subflavus* would be active within the hibernaculum all winter (Bernard and McCraken 2017) but predicted that activity would be higher during early-winter (Oct – Nov) than the other seasons (Berkova and Zukal 2006, Parsons et al. 2003). The results of this study could inform researchers about how environmental factors could influence *P. subflavus* activity within a WNS-positive site and how warmer temperatures could potentially affect winter activity.

2. Materials and Methods

2.1 *Study Area*

We monitored bat activity at Stumphouse Tunnel (hereafter referred to as Stumphouse), located in Oconee County, South Carolina (Fig. 1). The most common species found in Stumphouse was *P. subflavus* with historical populations of 100-300 individuals but, other species such as big brown bat (*Eptesicus fuscus*) and Rafinesque's

big-eared bat (*Corynorhinus rafinesquii*) were also found in small numbers. Stumphouse, an abandoned railroad tunnel, is 493 m long, 5.2 m wide and 7.6 m high. Approximately halfway through the tunnel in section B, there was a 4.9 m wide x 6.1 m long airshaft that extended 18.3 m up to the surface (Oconee County 2018), creating constant airflow into Stumphouse (Fig 2). *P. subflavus* primarily roosted in chamber C where hibernaculum temperatures and humidity remained constant. Prior to the arrival of WNS, Stumphouse housed over 300 *P. subflavus*. However, since the emergence of WNS in 2014-15, the population has declined by 91% (Loeb, unpublished data).

2.2 *Field Methodology*

We acoustically monitored bat activity at Stumphouse during late October 2016-March 2017 and late October 2017 – March 2018. We used four Anabat Roost Loggers (Titley Scientific) to monitor bat activity within Stumphouse. Roost Loggers record bat echolocation calls and have a narrow frequency range centered around 42 kHz and can therefore record *P. subflavus*, which have echolocation calls with a lower frequency of ~40 kHz. Because WNS-affected individuals are active during the day (Bernard and McCraken 2017), we programed the Roost Loggers to record continuously throughout day and night.

We placed Roost Loggers on the ground or on a stool with the microphone pointed toward the ceiling of the tunnel. Roost Loggers were spaced approximately 61 m apart in Stumphouse, one in chamber B and three in chamber C, since the latter housed the majority of *P. subflavus* (Fig 2). We replaced batteries every 3 months but otherwise

left equipment undisturbed. At the end of the hibernation season, we downloaded the data, filtered out all files containing only noise, and manually vetted files using AnalookW program. We identified calls to species using call characteristics such as slope, minimum frequency, and maximum frequency described by Corben (2002) and Britzke et al. (2002) and defined bat passes as $\geq$ 2 pulses in each file (Weller and Baldwin 2012).

We recorded barometric pressure (BP) every 30 minutes with a HOBO Micro Station Data Logger and Onset Barometric Pressure Smart Sensor. We placed one Micro Station in chamber C in 2016 and one Micro Station in chamber B and C in 2017. Micro Stations were deployed from November 2016 through March 2017 and from October 2017 through March 2018. We recorded hibernaculum temperature (T_H) and hibernaculum relative humidity (RH) using Hygrochron iButtons (Maxim Integrated, San Jose CA USA) every 30 minutes. We placed two iButtons in chamber B and three in Chamber B spaced evenly across Stumphouse Tunnel. We gathered hourly ambient temperature (T_A) from the nearest Remote Automated Weather Station (RAWS) weather station (Mesowest 2018) located near the study site. Full description of variables can be found in Table 1.

2.3 *Statistical Analyses*

The total number of bat passes per hour was used to quantify bat activity. This index does not equate to the number of *P. subflavus* that were active in Stumphouse but represents general activity. We did not include *E. fuscus* and *C. rafinesquii* calls in our

analysis since we were only interested in examining *P. subflavus* activity. We averaged iButton data in each chamber (B and C) to estimate hourly T_H and RH. We also calculated mean hourly BP and T_A. We combined data across years to look at seasonal variation.

We hypothesized that BP would influence hourly bat activity. Because Bender and Hartman (2015) found a positive relationship between mean nightly BP and nightly activity we predicted that activity would increase with BP. Studies such as Meyer et al. (2016) found positive correlations between activity and T_A. Although they examined bat emergence, we predicted that activity would increase with increasing T_A within a hibernaculum. Even though temperatures and RH within chamber C of Stumphouse were relatively constant, we hypothesized that T_H and RH would have an effect on activity. *P. subflavus* in Stumphouse are exposed to WNS and could react to small changes within the hibernaculum. Therefore, we predicted that lower RH would increase activity (Cryan et al. 2010) because during winter hibernation, individuals would prefer higher RH to decrease evaporative water loss during torpor (Thomas and Cloutier 1992) and lower RH could initiate activity. We also predicted that higher T_H would increase activity because cost of arousals would decrease with increasing T_H (Boyles et al. 2007). The primary drivers or cues for seasonal and annual activity patterns such as temperature (Parsons et al. 2003) and availability of resources (Wojciechowski et al. 2007) are well documented. Therefore, given the warmer climates of the southeast, we hypothesized that activity would vary seasonally and predicted that activity would be higher during early-winter (Oct-Nov) than mid (Dec-Jan) or late-winter (Feb - March). We categorized time of day

into three eight-hour blocks (0700 – 1400 early, 1500 – 2200 mid, 2300 – 0600 late) and because Stumphouse is a WNS positive site, we hypothesized activity could occur at any time of the day (Bernard and McCraken 2017).

Our data was not normally distributed therefore, we used generalized linear mixed models (GLMM) with a negative binomial distribution and accounted for zero-inflation in our count data using the glmmTMB package in R studio version 3.4.4 (R Core Development Team 2016). To account for two levels of temporal autocorrelation, we used a random effect of Julian day and offset Julian days as our blocking factor. We tested our environmental variables for multicollinearity using a correlation matrix and variables with an $|r| \geq 0.60$ were not included in the same model. We used an analysis of variance test (ANOVA) assuming equal variances to compare T_H, T_A, RH, and BP seasonally. We used a TukeyHSD test to compare levels of significance among seasons. We also used ANOVA to determine when bat activity was the highest both seasonally and during time of day. We used a TukeyHSD to compare levels of significance among seasons and time periods.

We used an information theoretic approach (Burnham and Anderson 2004) and developed two sets of models, one zero-inflated (Table 2) and one negative-binomial (Table 3). RH and T_H were not used in the same models because they were correlated. We tested nine hypotheses for our zero-inflated (zi) only model. We did not include a null model because zi models have limited scope and including a null only model will bias results towards the null hypothesis (Barry and Welsh 2002). We calculated Akaike Information Criterion (AICc) to evaluate support for the top zi model (Table 4). The zi

models were used to evaluate bat activity presence and we retained the top zi model to run in combination with the ten nb models. The negative binomial (nb) models were used to evaluate bat activity levels while considering bat presence. Model three did not converge and was not included in our final model set. The models we included represented environmental variables, seasonal variables, interactions, sub-global model, and an intercept only model. We used Akaike Information Criterion (AICc), to evaluate support for top models. Top models were assessed using ΔAIC values $\leq$ 2 (Posada and Buckley 2004). We model averaged if closely competing models existed and determined parameter significance within our top model by calculating upper and lower 95% confidence intervals and reported parameter estimates. We reported mean $\pm$ SD, test statistic, and *P*-values from our ANOVA and estimates from our GLMM.

3. Results

After manually vetting calls we recorded 1648 *P. subflavus* calls over 154 days (0.22 $\pm$ 1.02 calls per day) in 2016-2017 and 689 calls over 107 days (0.13 $\pm$ 9.67 calls per day) in 2017-2018. Roost loggers malfunctioned from Oct – Nov 2017; therefore, we did not collect bat activity data during that season. We also recorded 35 echolocation passes from other species in 2016-2017 and 96 echolocation passes from other species in 2017-2018. Echolocation passes from other species were only present in chamber B (roost logger #1) and accounted for 2.12% of all passes in 2016-2017 and 13.93% of all passes in 2017-2018.

3.1 *Cave Microclimate*

Ambient temperatures varied seasonally (F = 1212, df = 2,12485, P <0.0001) and T_A in each season was significantly different from the other seasons (Table 5). Mean T_A was the highest during early-winter and lowest during mid-winter. Hibernaculum temperatures also varied seasonally (F = 926.9, df = 2,11940, P <0.0001) (Table 5) and differed significantly among all seasons. Mean T_H was the highest during early-winter and lowest during mid-winter. Humidity significantly varied seasonally (F = 287.5, df = 2,11940, P < 0.0001) and RH in each season significantly differed from the others. Mean RH was the highest during late-winter and lowest during early-winter. While BP varied seasonally (F = 5.73, df = 2,8145, P < 0.003) only BP in early-winter (Oct – Nov) and mid-winter (Dec – Jan) significantly differed (Table 5). Mean BP was highest during mid-winter and lowest during early-winter.

3.2 *Activity Patterns of P. subflavus*

Although we recorded *P. subflavus* calls throughout the hibernation season, activity was low. Bat activity averaged $\leq$ 0.30 calls per day and average number of bat passes was highest during early-winter (Oct – Nov) (Table 5). Bat activity differed seasonally (F = 25.71, df = 2, P <0.0001) and differed significantly among all seasons (Table 5). *P. subflavus* activity varied significantly among time blocks (F = 11.45, df = 2, P < 0.001). Activity varied significantly between early (0.24 $\pm$ 1.06), and mid-day (0.16 $\pm$ 0.89, P = 0.0002,), but not between mid to late-day (0.16 $\pm$ 0.85, P = 0.97). Bat activity was highest later in the day during early winter (Fig 3) contrasting to late-winter when

bat activity was highest earlier in the day (Fig 4). Bat activity varied the most during mid-winter (Fig 5).

Our mixed model analysis showed that T_H, T_A, and season affected activity within Stumphouse Tunnel. The top zi model contained the environmental variables T_H, T_A, BP, season, and time (Table 6). Time, T_H, and T_A were important parameters in our top model and had 95% confidence intervals that excluded zero (Table 7). Presence of bat activity was higher in mid-day and late-day than early-day. Presence of activity was also positively correlated with T_A. Conversely, there was a negative relationship between T_H and bat activity presence. We did not find any significant relationships between season and BP for presence of bat activity in our zi models.

Environmental variables and season were important factors in predicting bat activity levels in Stumphouse (Table 6). Season and T_H were important parameters in our top model that best predicted activity levels of bats (Table 7). Activity levels varied among the seasons and levels were low during mid-winter and late-winter compared to early winter. Similar to our predictions, as T_H increased, level of activity within Stumphouse increased. Even though there was evidence to support our predictions that increasing T_H increased activity, the correlation was small ($R^2 = 0.01$). We did not find any significant relationship between T_A and BP on levels of bat activity in our top nb model.

4. Discussion

By examining acoustic data from a known WNS positive hibernaculum, we have demonstrated that T_H, T_A, and time of day best predict presence of bat activity within a hibernaculum and T_H and season best predict levels of *P. subflavus* activity. We determined there was no relationships between BP or RH and activity. Both BP and RH within Stumphouse had narrow ranges and we believe that the changes were either too small for *P. subflavus* to detect or the variables were not biologically relevant to individuals for activity. However, our study provides baseline data on winter activity patterns of *P. subflavus* in a WNS positive site.

4.1. *Microclimate and Activity*

P. subflavus have been observed to go into prolonged torpor at Stumphouse tunnel (Chapter 1) and through the use of Roost Loggers we were able to determine that *P. subflavus* were active within Stumphouse as well. We found a positive relationship between T_A and bat activity presence and studies such as Meyer et al. (2016) and Whitaker and Rissler (1992) have found similar results. Although, these studies primarily analyzed activity outside the roost, increasing T_A has often been associated with increased foraging (Park et al. 2000). BP has been associated with insect activity and bat emergence (Meyer et al. 2016) but based on our results it does not impact *P. subflavus* activity within Stumphouse. The effect of BP on hibernating bats is understudied and it is difficult to determine whether bats in deep torpor during winter were able to detect small

changes in BP or if a certain amount of change is required to initiate the physiological mechanisms for activity.

We found a negative relationship between T_H and presence of bat activity within Stumphouse. Presence of bat activity increased with decreasing T_H which could indicate that individuals could be moving within the roost to find more suitable roost conditions (Twente 1955). Conversely, we found a positive relationship between T_H and levels of bat activity. This positive relationship suggests that bats were able to detect small temperature changes within the hibernaculum and thus were more likely to be active when T_H is higher. This could be due to the fact that it is energetically more efficient for bats to arouse in warmer temperatures than in colder temperatures (Boyles et al. 2007) because arousals are energetically expensive (Thomas et al. 1990). Bat activity was not related to RH. RH is associated with evaporative water loss (EWL) and it is more energetically beneficial for an individual to arouse and remain active during higher RH to decrease water loss and dehydration (Thomas and Cloutier 1992). However, variation in RH was low and was possibly not detected by individuals within the tunnel.

4.2 *Seasonality and Time of Day*

Bats are more active during swarming (when bats enter the hibernation site) (Parsons et al. 2003) than when they exit the hibernaculum in spring. Even though Stumphouse might not be a swarming site, bat activity was the highest during early winter when individuals entered the hibernation site. Activity was the lowest during late-winter. *P. subflavus* could have exited the site in late-winter and not returned or switched

to different roost sites (Whitaker and Rissler 1992). WNS could be another factor for decreased activity during mid to late season. Even though we are not certain if all individuals at Stumphouse had WNS, we saw physical evidence of the disease on a few individuals particularly in late winter (Chapter 1, Appendix 1). We detected much lower activity during late winter when individuals displayed signs of WNS. Infected individuals are hypothesized to have more frequent arousals (Reeder et al. 2012) but they might not be active or echolocate after each arousal. We did not see evidence of individuals leaving the hibernaculum (Chapter 1) which suggests individuals either conserved energy to offset physiological costs of the disease by reducing activity after arousals or drank water that was found inside the tunnel. Stumphouse is a well lit site and contains a lot of water and individuals might not need to echolocate to find the nearest water source.

We did not find strong support for our hypotheses that levels of bat activity increased during early or late day to follow a circadian cycle but found support that bat activity presence was higher in mid – late day. This result may be due to how we divided the hours of the day. Bat activity presence was high during late day which is from 1600 hrs – 2300 hrs, suggesting bat activity followed the circadian cycle (Park et al. 2000, Turbill and Geiser 2008); however, if bats were active at 0000 hrs to sunrise then it would be considered early and not late. However, when we reclassified the time blocks into four and included blocks that were only sunset and sunrise hours, the models did not converge. This suggests that there is underlying collinearity in our variables that the correlation matrix did not pick up or our method to analyze bat passes hourly was not the best approach to examine environmental factors on bat activity.

4.3 *Conclusions*

Although activity was low, we detected the highest activity during early winter and the lowest during late winter. Even though there was no evidence of bats leaving the site, the seasonal variation in activity suggest that *P. subflavus* were more active when T_H was the highest during early winter. The environmental variables T_H and T_A were significant predictors of the presence of activity, while T_H was the only variable that predicted levels of activity within Stumphouse. Although there were conflicting relationships of T_H on activity, this suggests that *P. subflavus* are able to detect changes of T_H inside a tunnel. Climate change is another factor that should be considered. Based on our results, as ambient temperatures increase, the occurrence of activity also increases. Climate change could cause an increase in hibernaculum temperatures at Stumphouse which could potentially increase activity levels. Although we are unable to provide data on how much energy *P. subflavus* lost while active in Stumphouse, we know that arousals are energetically expensive and if activity increases due to climate change, individuals could expend more energy and lose fat stores more quickly. This could be problematic for individuals who are already constrained from the negative impacts of WNS.

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Table 1. Description of covariates used in both zero-inflated and negative binomial models to predict activity of bats in Stumphouse Tunnel, South Carolina, October-March 2017 and 2018.

Covariates	Description
Hibernaculum Temperature (T_H)	Hourly mean hibernaculum temperature recorded using iButtons at Stumphouse Tunnel, South Carolina. IButtons were programmed at 30-minute intervals.
Ambient Temperature (T_A)	Hourly mean ambient temperature downloaded from the RAWS weather station closest to Stumphouse Tunnel, South Carolina.
Humidity (RH)	Hourly mean humidity recorded using iButtons at Stumphouse Tunnel, South Carolina. IButtons were programmed at 30-minute.
Barometric Pressure (BP)	Hourly mean barometric pressure recorded using a barometric pressure sensor and hobo datalogger at Stumphouse Tunnel. Barometric pressure was recorded at 30- minute intervals.
Season	Season: early (October – November), mid (December – January), and late (February – March) during 2016 – 2018.
Time	Time of day: early (0000– 0700 hrs), mid (0800 – 1500 hrs), and late (1600 – 2300 hrs).

Table 2. List of models and covariates for zero-inflated mixed models used to predict bat activity presence at Stumphouse Tunnel (2016-2018).

Model	Zero-inflated
1	TH + TA + BP
2	Humid + TA + BP
3	Season + Time
4	Season + TH + TA + BP
5	Season + Humid + TA + BP
6	Time + TH + TA + BP
7	Time + Humid + TA + BP
8	Season + Time + TH + TA + BP
9	Season + Time + Humid + TA + BP

Table 3. List of models and covariates for negative binomial mixed models used to predict bat activity at Stumphouse Tunnel (2016-2018).

Model	Negative binomial
1	TH + TA + BP
2	Humid + TA + BP
3	(Season x Time)
4	Time + TH + TA + BP
5	Time + Humid + TA + BP
6	Season + TH + TA + BP
7	Season + Humid + TA + BP
8	(Season x Time) + TH + TA + BP
9	(Season x Time) + Humid + TA + BP
10	Intercept

Table 4. Zero-inflated models and akaike information criterion values used to predict bat activity at Stumphouse Tunnel, South Carolina (2016 – 2018).

Zero-inflated Models	Negative binomial	AICc	Delta AIC	W*i*
Season + Time + TH + TA + BP	~	13696.8	0.00	1.00
Season + TH + TA + BP	~	13714	17.20	0.00
Time + TH + TA + BP	~	13715.8	19.00	0.00
TH + TA + BP	~	13734.4	37.60	0.00
Season + Time + Humid + TA + BP	~	13829.5	132.70	0.00
Season + Humid + TA + BP	~	13843.6	146.80	0.00
Time + Humid + TA + BP	~	13907.9	211.10	0.00
Humid + TA + BP	~	13922.7	225.90	0.00
Season + Time	~	19802.9	6106.10	0.00

Table 5. Mean ± SD number of bat calls per day, hibernaculum temperature, ambient temperature, humidity, and barometric pressure in Stumphouse Tunnel, South Carolina October-March 2017-2018. Significant differences from the TukeyHSD test are indicated by letters within columns. Letters that are the same indicate no significant differences.

Season 2016 - 2018	Number of Bat Calls Per Season	Mean # Bat Calls Per Day	Mean Hibernaculum Temp (°C)	Mean Hibernaculum Humidity(%)	Mean Ambient Temp (°C)	Mean Barometric Pressure (Hg)
Early-Winter	542	0.30 ± 1.32 (a)	13.42 ± 2.11 (a)	89.8 ± 11.31 (a)	13.82 ± 5.54 (a)	28.55 ± 0.16 (a)
Mid-Winter	1221	0.21 ± 1.00 (b)	10.1 ± 3.36 (b)	90.6 ± 9.19 (b)	6.66 ± 6.41 (b)	28.6 ± 0.21 (b)
Late-Winter	573	0.12 ± 0.63 (c)	11.5 ± 2.23 (c)	94.5 ± 8.32 (c)	11.0 ± 6.04 (c)	28.6 ± 0.191 (a)

Table 6. Akaike information criterion values and model weights used to predict bat activity from zero-inflated and negative binomial mixed models at Stumphouse Tunnel (2016-2018).

Zero-inflated	Negative binomial	AICc	Delta AIC	W*i*
Season + Time + TH + TA + BP	Season + TH + TA + BP	13191.3	0.00	0.97
Season + Time + TH + TA + BP	Season + Humid + TA + BP	13198.4	7.10	0.03
Season + Time + TH + TA + BP	(Season x Time) + TH + TA + BP	13210.3	11.0	0.00
Season + Time + TH + TA + BP	(Season x Time) + Humid + TA + BP	13210.3	19.0	0.00
Season + Time + TH + TA + BP	TH + TA + BP	13558.4	367.10	0.00
Season + Time + TH + TA + BP	Time + TH + TA + BP	13560.1	368.80	0.00
Season + Time + TH + TA + BP	Intercept	13576.7	385.40	0.00
Season + Time + TH + TA + BP	Humid + TA + BP	13579.8	388.50	0.00
Season + Time + TH + TA + BP	Time + Humid + TA + BP	13583.6	392.30	0.00

Table 7. List of zero-inflated and negative binomial covariates with 95% confidence intervals and estimates from the top model.

Covariate	Estimate	2.5%	97.5%
Zero-inflated			
Intercept	-20.45	-8017.18	7976.28
Season: Mid-Winter	21.13	-7975.59	8.02
Season: Late-Winter	21.66	-7975.07	8.02
Time: Mid	0.40	0.05	0.73
Time: Late	0.87	0.53	1.21
TH	-2.24	-2.59	-1.88
TA	1.93	0.03	0.35
BP	-0.06	-0.20	0.08
Negative binomial			
Intercept	$-2.02x^{+6}$	$-2.01x^{+6}$	$-2.01x^{+6}$
Season: Mid-Winter	-11.12	-19.07	-3.23
Season: Late-Winter	-696.89	-707.83	-685.94
TH	29.05	13.83	44.18
TA	-1.04	-4.84	2.77
BP	1.82	-2.16	5.80

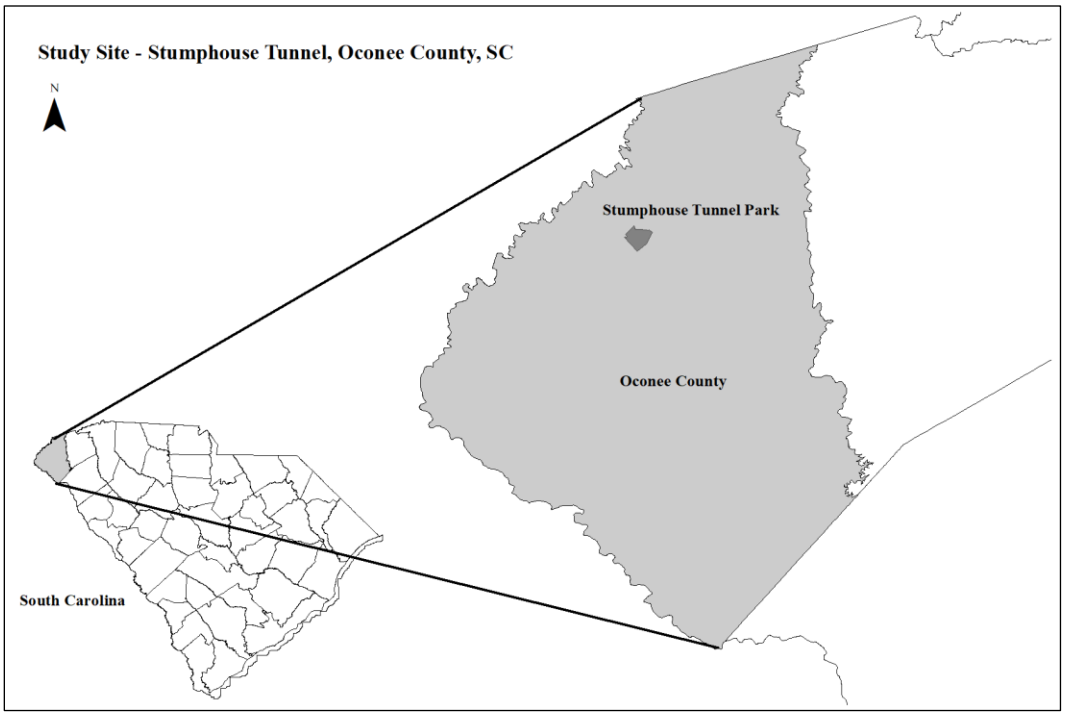

Figure 1. Study area: located at Stumphouse Tunnel Park, South Carolina, in the northwest park of the state. We collected data from October 2016 – March 2018.

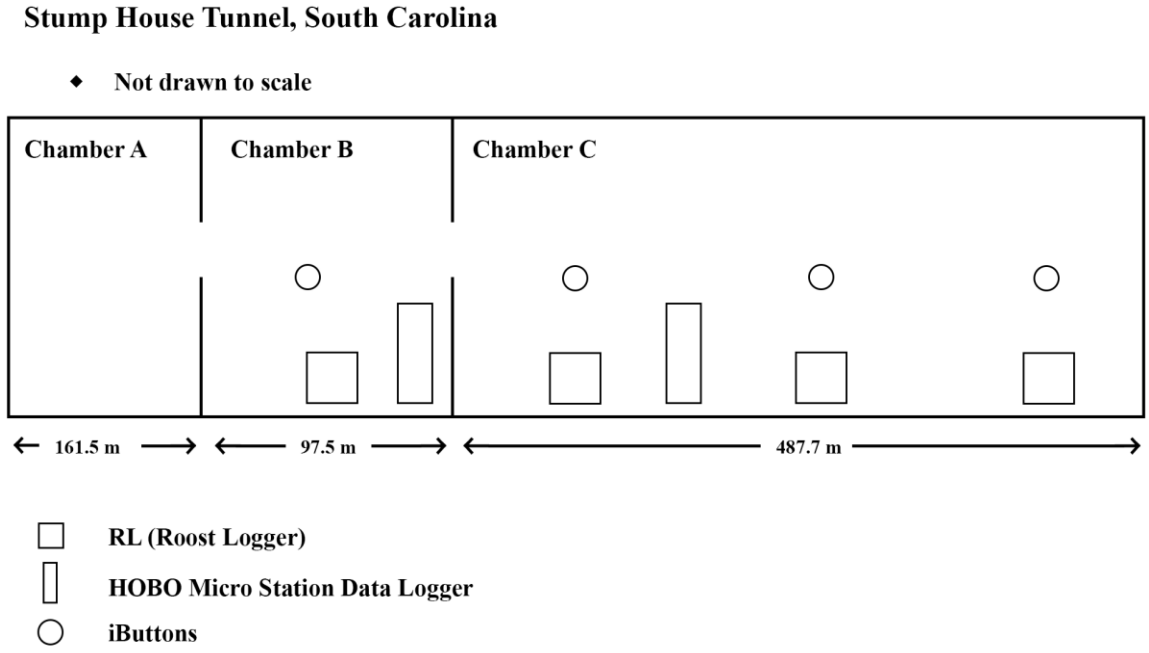

Figure 2. Schematic of study design at Stumphouse Tunnel. We added an additional Hobo micro station in October 2017 and placed it in Chamber B.

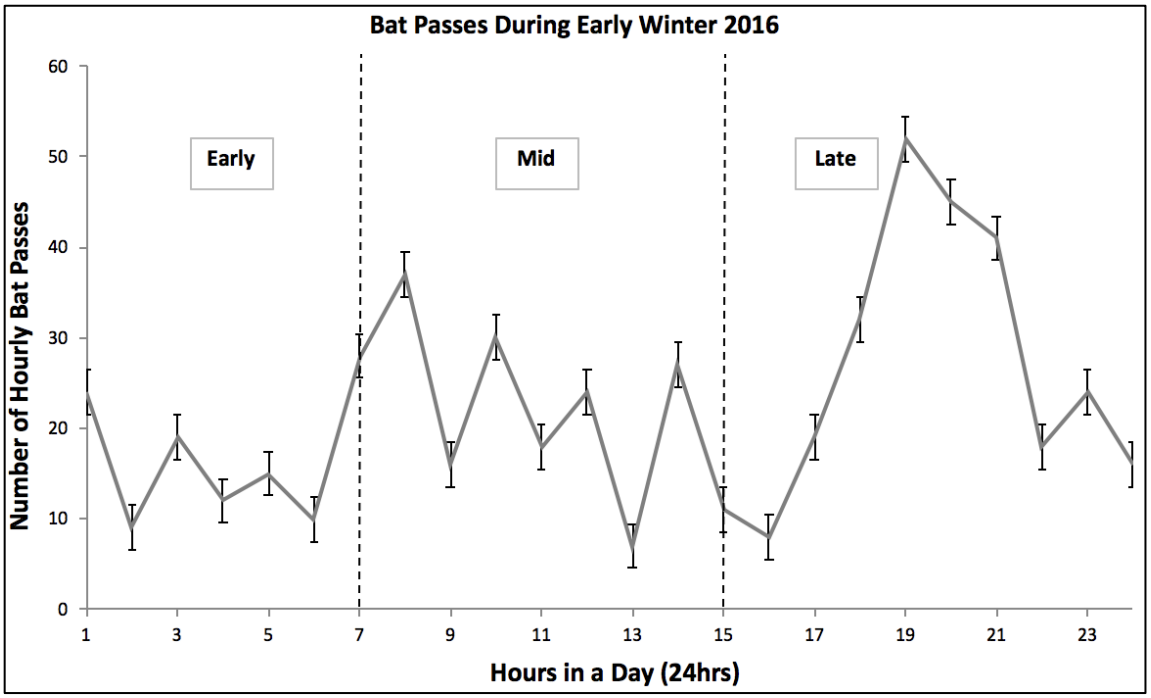

Figure 3. Bat activity during early winter (Oct – Nov) of 2016. Ticks represent standard error of bat passes for that hour.

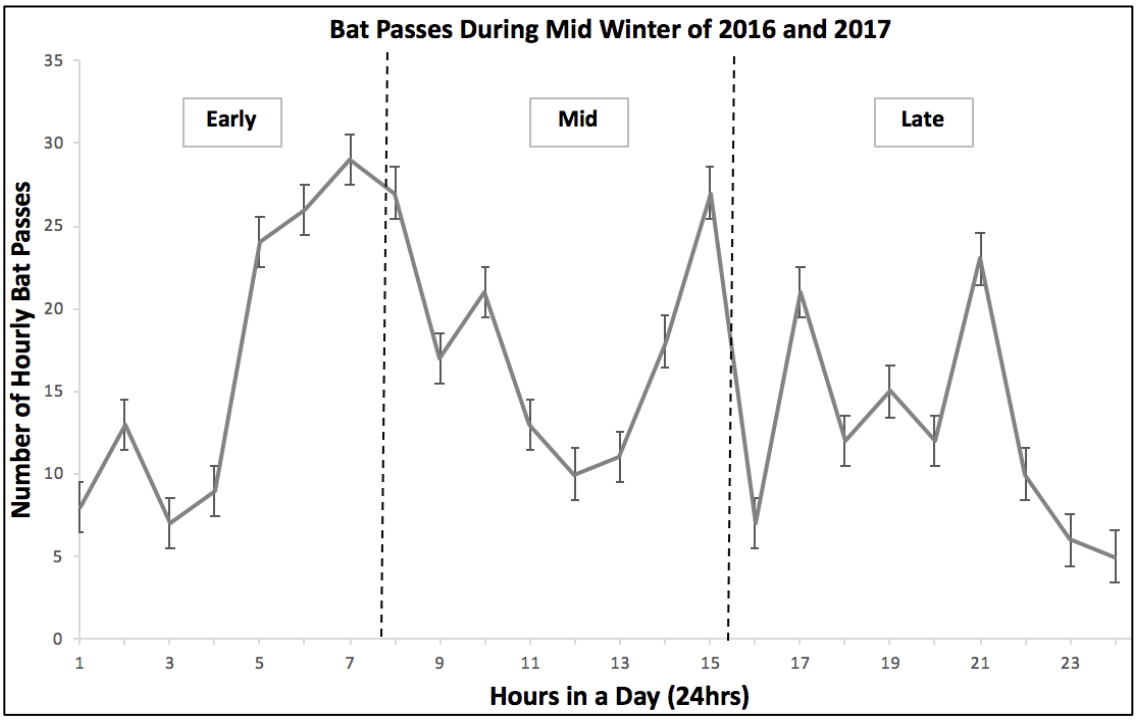

Figure 4. Bat activity during mid-winter (Oct – Nov) of 2016 and 2017. Ticks represent standard error of bat passes for that hour.

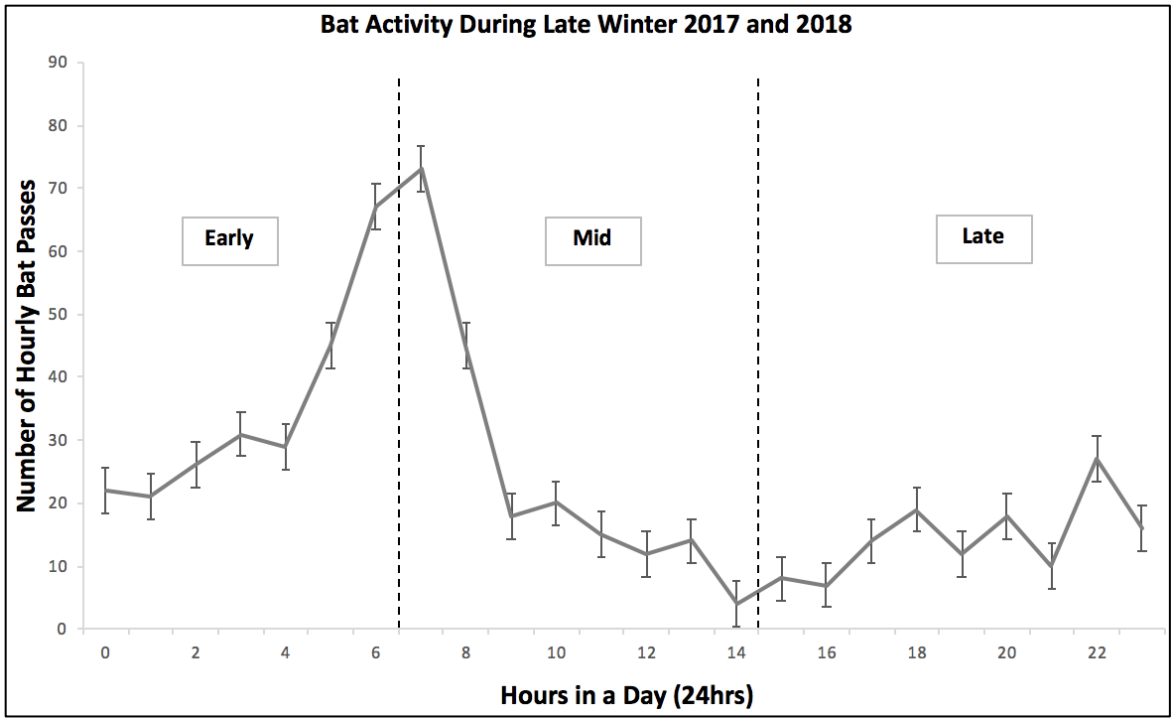

Figure 5. Bat activity during late-winter (Feb – March) of 2017 and 2018. Ticks represent standard error of bat passes for that hour.

Appendix – *Perimyotis subflavus* capture data from Florida, Mississippi, and South Carolina between 2016 – 2018. Includes sex, weight (g), forearm (FA) (mm), wing damage index, band number, recapture, and comments. Asterisks denotes missing information from data sheets.

Date	ID	Sex	Weight (g)	FA (mm)	Wing Damage Index	Band #	Site	Recapture	Comments
1/12/2017	1	F	6.5	33.8	0	FWC0703	Florida		
1/12/2017	2	F	5.75	33.8	0	FWC0704	Florida		
1/12/2017	3	F	6	33.6	0	FWC0705	Florida		
1/12/2017	4	F	7	25.4	0	FWC0706	Florida		
1/12/2017	5	M	5.75	34.9	0	FWC0707	Florida		
1/12/2017	6	F	6.5	35	0	FWC0708	Florida		
1/12/2017	7	M	5	33.3	0	FWC0709	Florida		
1/12/2017	8	F	6.75	35.6	0	FWC0710	Florida		
1/12/2017	9	M	5.75	35.1	0	FWC0711	Florida		
1/12/2017	10	F	7.25	34.1	0	FWC0712	Florida		
1/12/2017	11	F	6.5	35	0	FWC0544	Florida	Yes	Old tag removed (0542)
1/12/2017	12	F	4.5	34.2	0	FWC0713	Florida		
1/12/2017	13	M	5.1	33.4	0	FWC0714	Florida		
1/12/2017	14	M	5	34.1	0	FWC0740	Florida		
1/12/2017	15	M	4.75	33.3	0	FWC0741	Florida		
1/12/2017	16	M	5.5	34.4	0	FWC0742	Florida		
1/12/2017	17	M	5	32.7	0	FWC0750	Florida		
1/12/2017	18	M	5	34.3	0	FWC0747	Florida		
1/12/2017	19	M	5.5	33.9	0	FWC0748	Florida		
1/12/2017	20	M	5.5	34.3	0	FWC0744	Florida		
1/12/2017	21	M	5.5	34.9	0	FWC0716	Florida		
1/12/2017	22	F	6	34.2	0	FWC0743	Florida		
1/12/2017	23	M	5	34	0	FWC0715	Florida		

Date	ID	Sex	Weight (g)	FA (mm)	Wing Damage Index	Band #	Site	Recapture	Comments
1/12/2017	24	M	5.75	33.4	0	FWC0717	Florida		
1/12/2017	25	F	6.5	35.1	0	FWC0718	Florida		
*	26	M	5.25	33.7	0	A0024	Mississippi		
*	27	M	6.75	33	0	A0013	Mississippi		
*	28	M	5.25	33.2	0	A0053	Mississippi		
*	29	M	5	33	0	A0052	Mississippi		
*	30	M	5.25	32.5	0	A0051	Mississippi		
12/20/2016	31	M	7	32	0	A0098	Mississippi		
12/20/2016	32	F	5.5	31	0	A0095	Mississippi		
12/20/2016	33	F	6.5	32.5	0	A0097	Mississippi		
12/20/2016	34	F	7	33	0	A0096	Mississippi		
2/6/2017	35	F	6.5	33	0	A0089	Mississippi		
2/6/2017	36	M	5.8	32	0	A0090	Mississippi		
2/6/2017	37	M	7.5	32	0	A0093	Mississippi		
2/6/2017	38	F	7.5	33	0	A0094	Mississippi		
2/6/2017	39	F	5.7	32	0	A0091	Mississippi		
2/6/2017	40	F	7.1	34	0	A0092	Mississippi		
3/3/2017	41	M	7.25	31	0	*	Mississippi		
3/3/2017	42	F	6.5	32	0	*	Mississippi		
3/3/2017	43	M	5.75	31	0	*	Mississippi		
3/3/2017	44	M	6	32	0	*	Mississippi		
3/3/2017	45	M	6.25	32	0	*	Mississippi		
3/3/2017	46	M	6.25	31	0	*	Mississippi		
3/3/2017	47	F	6.75	33	0	*	Mississippi		
3/3/2017	48	M	6	33	0	*	Mississippi		

Date	ID	Sex	Weight (g)	FA (mm)	Wing Damage Index	Band #	Site	Recapture	Comments
1/19/2018	49	F	6.25	33	0	A0584	Mississippi		
1/19/2018	50	M	5	*	0	A0600	Mississippi	Yes	
1/19/2018	51	M	5.5	33.3	0	A0585	Mississippi		
1/19/2018	52	M	5.25	28.2	0	A0085	Mississippi		
1/19/2018	53	F	6.25	34.6	0	A0589	Mississippi	Yes	
1/19/2018	54	M	5	33.5	0	A0586	Mississippi		
1/19/2018	55	M	5	32.8	0	A0587	Mississippi		
1/19/2018	56	M	5.5	34.5	0	A0588	Mississippi		
1/19/2018	57	M	5	32.8	0	A0591	Mississippi		
1/19/2018	58	F	6.5	33.5	0	A0590	Mississippi		
1/19/2018	59	F	6.25	36	0	A0592	Mississippi		
2/5/2018	60	F	6	34.5	0	A0579	Mississippi		
2/5/2018	61	M	5.5	35.8	0	A0580	Mississippi		
2/5/2018	62	M	5	33.4	0	A0593	Mississippi		
2/5/2018	63	M	*	*	0	A0087	Mississippi	Yes	
2/5/2018	64	F	5.75	33.1	0	A0581	Mississippi		
2/5/2018	65	M	5.5	35.5	0	A0582	Mississippi		
2/5/2018	66	F	5.5	34.2	0	A0583	Mississippi		
2/5/2018	67	M	5	36	0	A0578	Mississippi		
2/5/2018	68	M	5.75	33	0	A0576	Mississippi		
2/5/2018	69	M	4.75	32.7	0	A0577	Mississippi		
12/19/2017	70	F	*	34	0	A0600	Mississippi		
12/19/2017	71	M	*	34	0	A0599	Mississippi		
12/19/2017	72	F	*	36	0	A0598	Mississippi		
12/19/2017	73	M	*	33	0	A0597	Mississippi		

Date	ID	Sex	Weight (g)	FA (mm)	Wing Damage Index	Band #	Site	Recapture	Comments
12/19/2017	74	M	*	33	0	A0596	Mississippi		
12/19/2017	75	F	*	35	0	A0595	Mississippi		
12/19/2017	76	F	*	33	0	A0594	Mississippi		
12/18/2015	77	M	5	32	0	SR0007	South Carolina		
1/11/2016	78	M	4.25	33.5	0	SR0029	South Carolina		
1/11/2016	79	M	4.75	34	0	SR0008	South Carolina		
1/11/2016	80	M	5	33	0	SR0045	South Carolina	Yes	
2/1/2016	81	M	4.5	33.2	0	SR0009	South Carolina		Small pin holes on R wing
2/8/2016	82	F	5.5	33	0	SR0010	South Carolina		Fungus on snout and genitals
2/8/2016	83	F	6.25	35.7	0	SR0011	South Carolina		
2/8/2016	84	M	6.5	34.5	0	SR0012	South Carolina		
2/22/2016	85	M	5	34	0	SR0013	South Carolina		Fungus around chin
2/29/2016	86	F	4.75	34	0	SR0014	South Carolina		Fluoresced on foot
11/15/2016	87	M	6.25	34	0	SR0800	South Carolina		
11/15/2016	88	M	6	33	0	SR0799	South Carolina		
11/15/2016	89	M	6.75	34	0	SR0798	South Carolina		
11/28/2016	90	F	6.5	34	0	SR0797	South Carolina		
11/28/2016	91	M	6.25	33	0	SR0156	South Carolina		
11/28/2016	92	M	5.75	35	0	SR0796	South Carolina		
12/12/2016	93	F	6.25	33	0	SR0794	South Carolina		
12/12/2016	94	M	4.75	33	3	SR0793	South Carolina		Left wing has a large portion missing
12/12/2016	95	M	6.5	34	0	SR0795	South Carolina		
1/2/2017	96	F	5.5	34	0	SR0792	South Carolina		
1/2/2017	97	F	6	34	0	SR0791	South Carolina		
1/2/2017	98	M	7	33	0	SR0790	South Carolina		
1/16/2017	99	F	4.75	33	0	SR0071	South Carolina		

Date	ID	Sex	Weight (g)	FA (mm)	Wing Damage Index	Band #	Site	Recapture	Comments
1/16/2017	100	F	5	34	0	SR0069	South Carolina		
1/16/2017	101	M	7.25	34	0	SR0012	South Carolina	Yes	
1/30/2017	102	M	4.25	33	0	SR0068	South Carolina		
1/30/2017	103	M	4.5	34	0	SR0051	South Carolina		
1/30/2017	104	M	5.25	34	0	SR0067	South Carolina		
2/20/2017	105	M	4.5	34	0	SR0066	South Carolina		Fluoresced on wings
2/20/2017	106	M	4.75	34	1	SR0065	South Carolina		Fluoresced on wings
2/20/2017	107	M	4.5	33	0	SR0156	South Carolina		Fluoresced on foot
2/27/2017	108	M	5.34	33	0	SR0019	South Carolina		Swabbed for different study
2/27/2017	109	M	5	33	0	SR0098	South Carolina		
2/27/2017	110	M	4.75	33	0	SR0053	South Carolina		
3/6/2017	111	F	5.5	34	0	SR0155	South Carolina		
3/6/2017	112	F	5	34	0	SR0097	South Carolina		
11/3/2017	113	F	6	33	0	SR0096	South Carolina		
11/3/2017	114	M	5.25	33	0	SR0054	South Carolina		
11/17/2017	115	F	5.5	32	0	SR0061	South Carolina		
11/17/2017	116	F	8.75	35	0	SR0056	South Carolina		
11/17/2017	117	M	6.25	34	0	SR0055	South Carolina		
12/1/2017	118	F	7	34	0	SR0079	South Carolina	Yes	
12/1/2017	119	M	6.25	33	0	SR0096	South Carolina	Yes	
12/8/2017	120	F	6.25	34	0	SR0094	South Carolina		
12/8/2017	121	M	5.25	33	0	SR0093	South Carolina		
12/15/2017	122	F	5.2	32	0	SR0057	South Carolina		
12/15/2017	123	M	5.75	33	0	SR0062	South Carolina		
12/15/2017	124	M	6.7	34	0	SR0058	South Carolina		
12/22/2017	125	M	5.75	32	0	SR0059	South Carolina		
12/22/2017	126	M	6	35	0	SR0796	South Carolina	Yes	

Date	ID	Sex	Weight (g)	FA (mm)	Wing Damage Index	Band #	Site	Recapture	Comments
12/29/2017	127	M	5.2	33	0	SR0060	South Carolina		